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Toll-like receptors in *Borrelia burgdorferi*-induced inflammation

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ABSTRACT

Lyme arthritis, the most common manifestation of late Lyme disease, has been associated with the presence of *Borellia burgdorferi* in the joint. However, it is still unclear whether the pathogen itself is able to elicit such a sustained inflammatory response, or whether an aberrant immunological reaction of the host is the main driving force. *Borrelia* antigens, including lipoproteins, flagellin and DNA, are ligands of Toll-like receptors, and can thus elicit a strong stimulation of host cells, such as neutrophils, mononuclear cells and resident tissue cells. Understanding the molecular basis of the signalling events caused by *Borrelia* lipoproteins will lead to a greater understanding of inflammation in Lyme arthritis and, hopefully, new treatment strategies for chronic antibiotic-resistant disease.

Keywords *Borrelia burgdorferi*, inflammation, review, spirochaetal persistence, tick-borne disease, Toll-like receptors

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INTRODUCTION

The term 'Toll' was first used to describe a cell-surface receptor governing dorsoventral axis polarity in early *Drosophila melanogaster* (fruit fly) larvae [1]. In addition to developmental roles, it became clear that Toll receptors play an essential role, together with other antimicrobial peptides, in the activation of non-specific antifungal defence mechanisms in the fly [2]. A comparable system of antimicrobial defence mechanisms has been identified in humans by studying lipopolysaccharide (LPS)–host interactions. These receptors have been termed 'Toll-like receptors' (TLRs). It is now known that TLRs play an important role in the signalling of many pathogen-derived molecules, and also in some endogenous proteins associated with activation of the immune system. To date, ten human and nine murine trans-membrane proteins have been reported to belong to the mammalian TLR family [3,4]. All TLRs are characterised by an extracellular domain (ectodomain) with leucine-rich repeats (LRR), and a single trans-membrane domain followed by an intra-cytoplasmic domain that is required for signal transduction. The intra-cytoplasmic domain is also called the TIR domain (TOLL/interleukin-1 receptor homology domain) on the basis of its homology with the interleukin-1 type-I receptor (IL-1RI) [5]. It has been demonstrated in various studies that *Borrelia*-derived antigens can interact with the TLR system and thereby stimulate the production of pro-inflammatory factors.

Borrelia burgdorferi, the spirochaete that causes Lyme disease, can invade and persist in multiple organs for long periods. Lyme disease is a multisystemic disorder, which involves primarily the joints, the heart, the central and peripheral nervous system, and the skin. The initial stages of Lyme disease are treated with antibiotics. However, if misdiagnosed or left untreated, the spirochaete may spread from a localised skin infection to other body parts and tissues. Treatment- or antibiotic-resistant Lyme borreliosis has been described in the later stages of the disease. In human and murine disease, the pathology of Lyme borreliosis is considered to be related to the

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presence of the bacterial pathogen in tissues, suggesting that either living or dead bacteria, or bacterial products, might be responsible for eliciting inflammation.

B. burgdorferi has been shown to produce a number of membrane-associated lipoproteins, and many of these are expressed preferentially during mammalian infection. Several of these lipoproteins have been shown to directly stimulate immunological effector cells responsible for mediating an innate immune response; such responses include induction of inflammatory mediators by macrophages [6], the expression of adhesion molecules and the production of superoxide by neutrophils [7], induction of NF-kB nuclear translocation and up-regulation of downstream inflammatory events in endothelial cells [8], activation of mast cells [9], and mitogenesis and immunoglobulin production in B-cells [6,10]. The inflammatory events induced by these lipoproteins suggest that persistence of *B. burgdorferi* in tissues could contribute to the tissue pathology of Lyme disease [11–13].

The role of TLR2 in *B. burgdorferi* infection has been studied extensively. This review describes the role of the major TLRs in host defence and, in particular, the role of TLR2 in relation to *B. burgdorferi*-induced inflammation.

EXPRESSION OF PATTERN RECOGNITION RECEPTORS AND PATHOGEN RECOGNITION

The innate recognition of pathogens is mediated initially by a set of germline-encoded receptors known as pattern recognition receptors (PRRs). These can directly recognise conserved molecular structures on pathogens, which are known as pathogen-associated molecular patterns (PAMPs). PAMPs are shared by groups of microorganisms [14]. There are only a few main features that characterise PAMPs: they are usually expressed by microbes, but not by host cells; they show little variation among microorganisms of a given class; their expression can be essential for the survival and pathogenesis of the microbe; and limited variation of the pathogen allows a class recognition of microbes, and prevents, in part, the development of mutants which could escape recognition by the host immune system [15].

It has been demonstrated that a range of bacterial molecules, such as hypomethylated DNA with CpG motifs, lipoteichoic acid, peptidoglycans, lipoarbinomannans, lipopeptides and choline-containing phosphoglycolipids, can interact with the mammalian innate immune system. Receptors of PAMPs include secreted PRRs (LPS-binding protein; LBP), cell surface PRRs (such as TLRs, CD14, the macrophage scavanger receptor and the mannose receptor), and intracellular PRRs (dsRNA-activated protein kinase) [15] (Table 1, Fig. 1 and Fig. 2).

The recent addition of TLRs to the list of cell-surface PRRs has opened a new field of research. TLR-family members are expressed by host cells involved in the first line of host defence, including neutrophils, macrophages, dendritic cells, mucosal epithelial cells [16] and synovial fibroblasts [17]. TLR2 and TLR4 are the major receptors for bacterial lipoproteins and LPS, respectively. Both receptors are also expressed on B- and T-cells, which mediate the complex adaptive immunity by a large repertoire of antigen-specific immunoglobulin class receptors [18]. Thus, the innate and adaptive branches of the host's immune system are well-connected. The innate system is thought to keep infectious pathogens at bay until the more specific adaptive response is mounted. With the exception of TLRs 3, 7, 8 and 9, which are intracellular receptors, TLRs are generally expressed on the cell-surface. The subcellular localisation of TLR4 differs in macrophages and intestinal epithelial cells. In macrophages, TLR4 is expressed on the cell-surface and is internalised following engagement of a ligand, whereas in epithelial cells it resides in the Golgi apparatus [16,19].

ROLE OF TLRs IN HOST IMMUNE DEFENCE

One of the distinctive features of the immune system is that it relies on cell migration for surveillance, attack, containment and clearance of invading pathogens. Two types of cell migration are used by the cells of the innate and adaptive immune systems, i.e., inducible and homeostatic cell migration. Inducible cell migration is generally triggered as the result of the sensing of pathogens through PRRs, as in neutrophil recruitment to the local site of infection. Steady-state or homeostatic cell migration allows naïve lymphocytes to circulate among the secondary lymphoid tissues throughout the body. Once an antigen is encountered via B- or T-cell-

Table 1. Toll-like receptors and their ligands

Toll-like receptor	Ligands	Origin of ligand, pathogens or disease
TLR1	• only signals as dimer when combined with TLR2 for all its ligands	
	• recognises *Borrelia burgdorferi* OspA, required for adaptive immune response	Lyme disease, *B. burgdorferi*
	• lipopeptides	
	• modulin	
TLR2	• associates with TLR1, TLR6	
	• lipoproteins/lipopeptides, *B. burgdorferi* OspA	Lyme disease, *B. burgdorferi*
	• peptidoglycan	
	• lipoteichoic acid	
	• lipoarbinomannan (mycobacterial),	*M. tuberculosis*
	phenol soluble modulin (*Staphylococcus epidermidis*),	
	glycoinositolphospholipids (*Trypanosoma cruzi*)	Chagas disease
	• glycolipids (*Treponenma maltophilum*)	
	• Porin (*Neisseria*)	
	• zymosan (yeast)	fungal sepsis
	• HSP70 (host)	
TLR3	• double stranded RNA	double stranded RNA viruses
TLR4	• Gram-negative enteric LPS (requires co-receptors MD-2, CD14)	Gram-negative bacteria
	• F protein	Respiratory syncytial virus
	• taxol	plant
	• *Chlamydia* HSP60	*Chlamydia trachomatis* and
	• HSP60 and HSP70	*Chlamydia pneumoniae*
	• polysaccharide fragments of heparan sulphate	host
	• fibrinogen	host
TLR5	• flagellin from bacteria	flagellated bacteria, Gram-negative bacteria and Gram-positive bacteria
TLR6	• soluble tuberculosis factor	
	• small antiviral compounds	
TLR7	• small antiviral compounds	viral infections
TLR8	• small antiviral compounds	viral infections
TLR9	• bacterial unmethylated DNA as CpG motif	bacterial and viral infections
TLR10	• expressed on B-Cells and plasmacytoid dendritic cells, but specific ligands are unknown	unknown

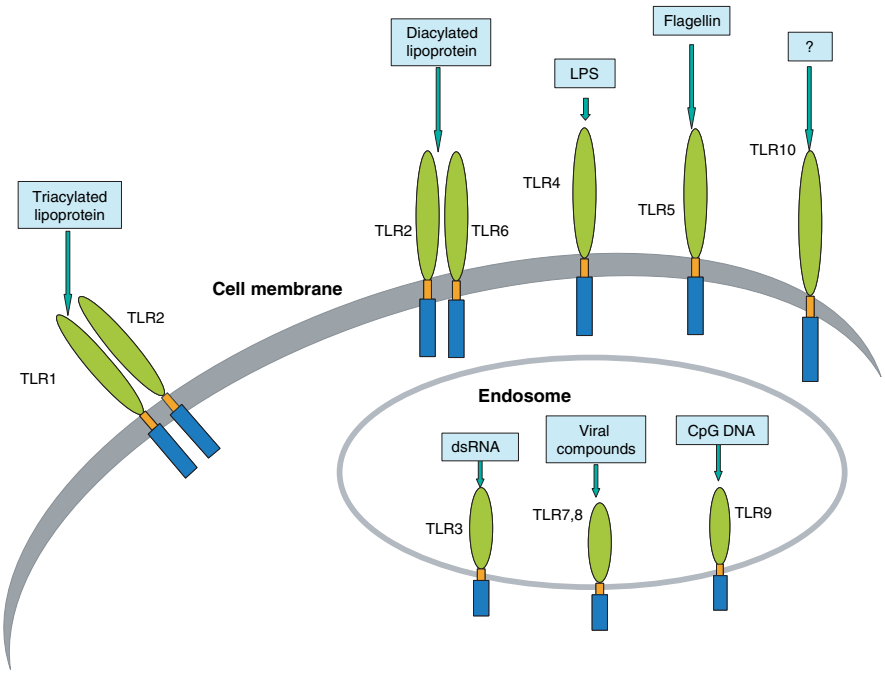

Fig. 1. Toll-like receptors and their ligands

specific receptors, an adaptive immune reaction might be mounted. This pathway is constitutive and does not require induction through TLRs [20].

Innate recognition of PAMPs through TLRs initiates an inflammatory response characterised by the recruitment of the cells to the site of infection. Cell migration from peripheral blood

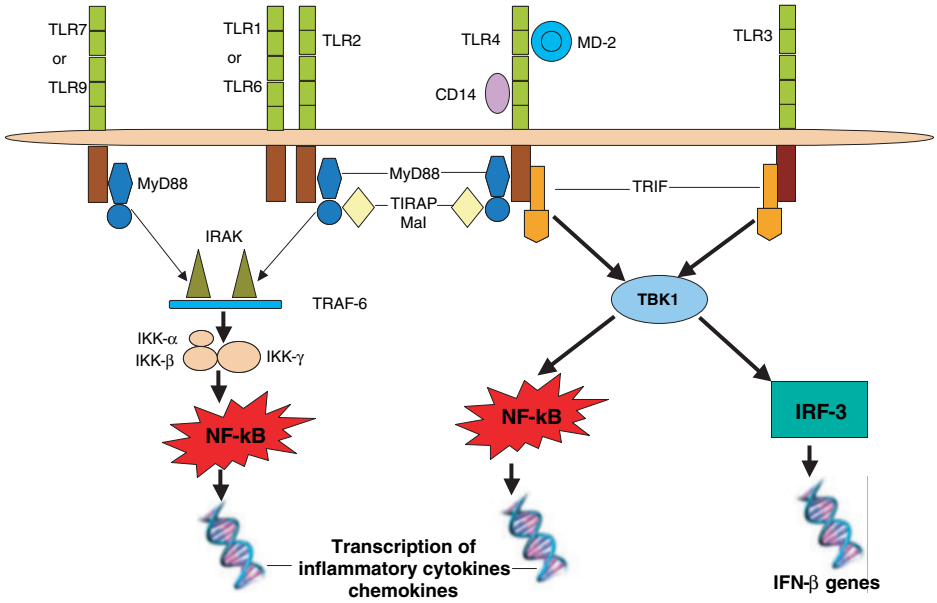

Fig. 2. Toll-like receptor (TLR) signalling pathways. Binding of a pathogen-associated molecular pattern (PAMP) to a particular TLR leads to the activation of TIR, and triggers a series of events leading to increased expression of pro-inflammatory genes. TLRs and IL-1Rs share a common adaptor molecule MyD88. After binding of a ligand, MyD88 is recruited to the TIR Toll/interleukin-1 receptor homologous region (TLR/TIR) receptor complex, which is then joined by IL-1R-associated protein kinase-1 (IRAK-1), IRAK-4 and tumour necrosis factor receptor associated factor 6 (TRAF6). IRAK-1 and TRAF-6 then dissociate from this complex and associate with another complex of transforming growth factor β-activated kinase (TAK-1) and TAK-1 binding proteins 1 and 2. TAK-1 is activated, which in turn activates the IκB kinase (IKK) complex. IKK-mediated phosphorylation of IκB leads to its ubiquitination and degradation, thereby unmasking the nuclear localisation domain of NF-κB [80]. After NF-κB translocation into the nucleus, NF-κB can activate multiple pro-inflammatory genes including TNF-α, IL-1 and IL-6 [80]. See main text for further explanation of abbreviations.

to the inflamed tissues is a multistep process [20]. Two types of signals mediate these steps, i.e., diffusible chemotactic factors and cell-surface adhesion molecules. Activation of cells by TLRs induces the expression of selectin, chemokine and chemokine receptor genes in local tissue cells and migratory immune effector cells that regulate cell migration to the sites of inflammation [21] (Fig. 2). In addition, TLR engagement initiates a signalling pathway that stimulates the cellular components of the host innate defence system and leads to an induction of reactive oxygen species and nitrogen intermediates [5]. It can also stimulate the first steps of the adaptive immune system by induction of pro-inflammatory cytokines and the up-regulation of co-stimulatory molecules in antigen-presenting cells. When sensing inflammatory signals, leukocytes rely first on vascular endothelial cells. This process is mediated by selectins expressed by the endothelium, and by carbohydrate ligands on leukocytes. Selectins are displayed rapidly on the endothelial surface, either after direct recognition of pathogens through TLRs, or by tumour necrosis factor secreted from activated tissue macrophages. Key inflammatory chemokines produced during microbial infection include interleukin-8 (IL-8/CXCL8), monocyte chemo-attractant protein-1 (MCP-1/CXCL1), MCP-2 (CCL8), macrophage inflammatory protein-1 α (MIP-1 α/CCL3) and RANTES (CCL5) [20,22]. Cell adhesion molecules are also induced directly by TLRs, or indirectly through tumour necrosis factor and IL-1, which are produced by TLR-activated resident tissue macrophages [23]. Differential expression of chemokines [24], adhesion molecules [12], matrix metalloproteinases [25] and cyclo-oxygenases has been demonstrated in synovial cells exposed to *B. burgdorferi* isolates Geho and B31 [25]. It may be that different *B. burgdorferi* isolates directly induce or signal through TLRs by different mechanisms, leading to a differential expression of molecules relevant for inflammation. This could be a major cause of the variation of inflammation in time and severity associated with Lyme disease.

The action of the innate immune system in peripheral tissues is thought to have a limited ability to eradicate pathogens in mammals [26]. A more effective host defence is achieved by the activation of adaptive immune responses, which mostly takes place in secondary lymphoid tissues such as lymph nodes [26]. Immature dendritic cells (DCs) play an essential role in the communication between the peripheral site of infection and these secondary lymphoid tissues [27]. In contrast to macrophages, which function mainly to eradicate pathogens, DCs are important for sensing the invading pathogen and for transferring the information to the adaptive immune system. In order to activate T-cells, DCs need first to leave the peripheral tissues and to migrate to regional lymph nodes [26]. In mice and humans, dendritic cell trafficking is mediated by chemokine receptors following stimulation of TLRs [28,29]. LPS, a typical PAMP interacting with TLRs, can down-regulate the expression of chemokine receptors, such as CCR5, and up-regulate CCR7 expression on DCs. TLR signalling stimulates the maturation of DCs. Thus, bacterial infection activates DCs and can stimulate them to produce mainly T_H1-inducing cytokines such as IL-12. Differentiation into T_H1 or T_H2 CD4 + effector T-cells [30] can be directed by DCs, depending upon the particular DC subsets, the DC maturation stage, or the DC to T-cell ratio [26,31].

Stimulation of T-cells is mediated by the presentation of MHC molecule-associated pathogen-derived peptides, combined with a variety of secondary signals delivered by co-stimulatory molecules such as CD80/CD86 [5,26]. T-cell differentiation is important for the establishment of an effective adaptive immunity [30]. T_H1 cells secrete mainly the effector cytokine IFN-γ and are involved in cellular immunity. T_H2 cells produce the effector cytokines IL-4, IL-10 and IL-13, and are thought to be involved in humoral immunity. Therefore, TLR-stimulated DCs tend to direct T-cell differentiation towards the T_H1 cell type. These observations show that TLRs are crucial, not only in the early phase of infection, but also in linking innate and adaptive immunity throughout the entire course of the host defence response.

MAMMALIAN TLRs AND THEIR LIGANDS

TLR1 and TLR2 are responsible for the response to triacylated bacterial lipoproteins [32,33]. Virus replication in infected cells results in the generation of dsRNA and induction of type-I interferon; dsRNA acts as a PAMP because it is not a constituent of host cells, and is recognised by TLR3 (Fig. 1) [34]. Most pathogenic and commensal bacteria produce flagellin. Flagellin is a monomeric structural subunit of bacterial flagella, and TLR5 is able to sense flagellin. Flagellin shows a potent pro-inflammatory activity by inducing IkB degradation, and thus NF-kB activation [35]. TLR6 and TLR2 recognise diacylated bacterial lipoproteins [36]. CpG oligonucleotides derived from bacterial DNA signal through TLR9 [37].

There is broad ligand specificity for different ligands recognised by different TLRs. How such different ligands, either proteins or lipids, can induce signals via the same TLR is still unclear. A set of TLRs can cluster to induce responses to a specific ligand [38]. It has been suggested that the ability of TLR2 to bind several ligands is based on its ability to form heterodimers with other TLRs, particularly TLR6 or TLR1 [38]. Thus, TLR2 appears to broaden its repertoire of specificities indirectly by forming at least two distinct types of functional heterodimers with other TLRs. This observation has been supported by a study of knock-out mice. It has also been demonstrated that TLR1 signalling is not necessary, but that it can enhance the stimulatory response to triacylated lipoproteins via TLR2. In contrast, TLR6 seems to be necessary for a host response to diacylated lipoproteins via TLR2 [36].

Proteins other than the TLRs themselves have been shown to be necessary for TLR binding and signalling. The small molecule MD-2 is required for responses to LPS via TLR4 [39], and may also form aggregates with TLR4 receptors [40]. The role of CD14 in binding a variety of microbial compounds, thereby enhancing activity for TLR ligands towards cellular activation, has also been established [41].

TLRs AND *BORRELIA BURGDORFERI*

The severity of clinical symptoms associated with bacterial diseases may vary according to the type and abundance of the infectious agent, the affected tissue, and co-existing illnesses. However, similar host responses have also been observed. For example, several clinical and immunological

similarities can be seen during infection with *Treponema* and *Borrelia* spp., and between Gram-negative and Gram-positive sepsis [42]. Hence, it can be speculated that the pathophysiological similarities observed with diverse infections are caused by activation of analogous signalling pathways in response to bacterial exposure.

Sensing of different pathogen-derived molecules via TLRs can mediate host inflammatory reactions to a range of microbial pathogens. Understanding the immunopathology of Lyme borreliosis is still a major challenge. Although *B. burgdorferi* can induce a strong bactericidal immune activation, e.g., in phases of arthritis, the causative agent of Lyme borreliosis seems to be able to persist in humans, which probably contributes to a chronic pathology in the immunocompetent host [11]. A remitting and episodic course of inflammation has been described, indicating a repetitive confrontation of the immune system with spirochaetal components [43]. The genome of *B. burgdorferi* contains *c.* 150 genes that encode putative lipoproteins. *B. burgdorferi* does not produce LPS [44], nor does its genome encode the enzymes required for LPS synthesis [45], but it manufactures abundant lipoproteins [46]. Thus lipoproteins seem to provide the major inflammatory stimulus associated with infection. The existence of a specific receptor for lipoproteins could provide a mechanism by which bacteria could activate inflammation directly. Bacterial lipoproteins and LPS share many characteristics, including a biologically active lipid modification, the types of cells that are responsive, and the types of responses that are elicited [47].

Spirochaetal lipoproteins and synthetic lipohexapeptide analogues are potent activators of monocytes/macrophages, neutrophils, lymphocytes, endothelial cells and fibroblasts [42]. The acyl modification of the peptides is essential for their pro-inflammatory activities. Strong pro-inflammatory capacities for the lipoproteins of *B. burgdorferi* were described first for outer-surface proteins A and B. Both are highly immunogenic [48] and have been the basis for the development of Lyme disease vaccines [49]. These compounds exhibit a triacylated lipid anchor structure, comprising an N-palmitoyl-S-[2,3-bis(palmitoyloxy)-(2RS)-propyl]-(R)-cysteinyl (Pam_3Cys) moiety at the N terminus [50], which is a feature that has also been described for lipoproteins from *Escherichia coli* [51]. A Pam_3Cys moiety has also been described in another spirochaete, *Treponema pallidum* [52], and has been reported to be present in cytokine-inducing lipoproteins from *Mycobacterium* and *Mycoplasma* spp. [53,54]. Thus, Pam_3Cys can be regarded as a conserved molecular motif among different classes of bacteria. The inflammatory activities attributed to *B. burgdorferi* lipoproteins include an ability to directly induce NF-κB nuclear translocation, resulting in cytokine production, adhesion molecule expression, and generation of nitric oxide and superoxide [55–58]. Both TLR2 and TLR4 are expressed on the surface of a wide variety of human cells [3,59], and have been reported to have a role in the recognition of bacterial lipoproteins and lipopolysaccharide, respectively, as well as in the induction of NF-κB and activator protein-1.

It has been demonstrated that defects in TLR1 and TLR2 can abrogate the response to OspA in OspA-vaccinated mice. It has also been reported that humans who are hypo-responsive to OspA vaccination have altered cell-surface expression of the TLR1 receptor, and may have a disturbance in the TLR1-mediated signalling pathway. These studies provide a link between TLR1, TLR2 and acquired humoral immune responses [33].

Wang *et al*. [60] performed a study of the impact of TLR2-mediated signalling on host defence and susceptibility to infection by using *B. burgdorferi* isolates RST1 and RST3A in TLR2-deficient mice. Mice infected with RST1 developed more severe arthritis and carditis compared with the mice infected with RST3, demonstrating that different genotypes of *B. burgdorferi* differ in their pathogenicity [61]. RST3A cells were more likely to be killed by the counter-attack of the host defence mechanism in the early stage of infection. In TLR2-deficient mice infected with RST1, the innate host defence system was shown to be impaired, which resulted in a significant increase in the number of spirochaetes in various tissues. Quantitative differences in spirochaete levels were thought to reflect the impairment of host immunity. Spirochaete dissemination would be limited under immunocompetent conditions, resulting in the clearance of bacteria from target tissues. However, despite significantly higher numbers of spirochaetes, TLR2-deficient mice developed a milder carditis than wild-type C3H/HeJ mice. This was considered to be evidence that TLR2-mediated signalling is involved in antibacterial action, and also in

pathological pro-inflammatory responses and disease evolution. During the course of infection, bacteria may stimulate specific cell-signalling receptors, resulting in a selective induction of host genes that may even facilitate bacterial invasion, colonisation and growth, supporting the inflammatory reaction. The TLR2 receptor is expressed on the surface of mammalian monocytes or macrophages [62]. The absence of TLR2-dependent signalling could result in disruption of the host inflammatory response mediated by macrophages. The latter might not be beneficial in every case.

It is possible that only a limited number of spirochaetes are required to initiate host inflammatory signalling in target tissues. Once the density of spirochaetes in joints reaches a particular threshold, pro-inflammatory signalling could be activated and may result in severe arthritis in mice, regardless of the number of spirochaetes. In the TLR2-deficient mouse, it is also possible that spirochaetes were cleared by other host defence mechanisms independent of the TLR2 pathway. TLR2-independent pathways could be mediated by non-lipoprotein components of spirochaetes, such as flagellin, CpG motif-containing DNA and glycolipids [4,60].

Several studies have demonstrated a critical role for *Borrelia*-specific immunoglobulin (Ig) in the protective host defence to *B. burgdorferi* [63–65]. *B. burgdorferi* lipoproteins have been reported to possess potent B-cell mitogenic properties that are capable of stimulating polyclonal activation, proliferation and Ig production *in vitro*. Increased numbers of *B. burgdorferi* spirochaetes in TLR2-deficient mice cannot be attributed to a defective humoral immune response, as anti-spirochaete IgG levels were normal. This suggests that effective elimination of *B. burgdorferi* requires not only specific anti-*Borrelia* Ig, but also TLR2-expressing effector cells. TLR2 is reported to be expressed in activated B-lymphocytes within germinal centres, but is not expressed by resting B-lymphocytes [59,66]. These results suggest that B-cell activation by lipoproteins may be subsequent to TLR2 engagement.

Neutrophils have been shown to play a critical role in spirochaete clearance [67], and may be very important in controlling numbers of Ig-opsonised *B. burgdorferi* in TLR2-deficient mice. It has been suggested that the lipoprotein–TLR2 interaction plays a crucial role in activating cells of the innate defence system, leading to phagocytosis and killing of spirochaetes. In addition, resident glial cells, including astrocytes and microglia, seem to play an important role in the host response to *B. burgdorferi* in neuroborreliosis. Microglia are resident immune cells of the central nervous system, and are descendents of the same cellular lineage as monocytes and macrophages. Rasley *et al*. [68] demonstrated that exposure of microglial cells to *B. burgdorferi* leads to an induction of TLR2 and CD14 genes.

One candidate for non-lipoprotein stimulatory activity in sonicated *B. burgdorferi* is unmethylated CpG DNA, which has been shown to activate murine cells through TLR9 signalling [37]. Additionally, flagella from two diverse bacteria, *Listeria monocytogenes* and *Salmonella* Typhimurium, have been reported to signal via TLR5, suggesting that *Borrelia* flagella are also able to stimulate via TLR5.

Metalloproteinases (MMPs) have been implicated in many inflammatory processes involving the nervous system and the joints, both of which are sites of injury in Lyme disease [25]. It has been demonstrated that secretion of MMP-9 is induced selectively by TLR2 in human and murine monocytic cells stimulated with *B. burgdorferi* [69]. Analysis of nuclear extracts indicated that activator protein (AP-1) plays a role in transcriptional activation of MMP-9 through TLR2 in monocytes. Secretion of MMP-1 was shown to be stimulated through a pathway other than one involving TLR2.

Arthritis is a frequent complication of Lyme borreliosis. Intermittent episodes of arthritis develop in *c.* 60% of individuals infected with *B. burgdorferi*, especially in North America [70,71]. In severe cases, chronic inflammatory Lyme arthritis involves cartilage and bone erosion, leading to permanent joint dysfunction [72,73]. Elevated expression of functional TLR2 has been reported in synovial fibroblasts of patients affected by rheumatoid arthritis [74,75], indicating that rheumatoid arthritis synovial fibroblasts respond to TLR2 ligands by up-regulation of various chemokines [17]. Our own studies have shown a differential expression of chemokines by human synovial cells exposed to different *B. burgdorferi* isolates. Thus, it is possible that different strains of *B. burgdorferi* can stimulate synovial cells through TLRs either directly or indirectly. This differential expression of chemokines, together

with other inflammatory factors, might contribute to the clinical variability in Lyme disease patients. It can be hypothesised that spirochaetal products, e.g., lipoproteins, flagellin or others, could be present in the joint as potential ligands of TLRs.

GENERAL PROPERTIES OF TLR SIGNALLING PATHWAYS

To a large extent, the specificities of the TLRs, and the way they signal, have now been deciphered. Four adaptor proteins, MyD88 (myeloid differentiation factor 88), MAL/TIRAP (MyD88-adaptor like/TIR associated protein), TRIF (Toll-receptor associated activator of interferon) and TRAM (Toll-receptor associated molecule), transduce signals from all of the TIR (Toll/interleukin-1 receptor (IL-1R) homologous region) domains, activating protein kinases and then the transcription factors that cause inflammatory effects [76]. MyD88 was isolated originally as a primary response gene in myeloid differentiation. It was induced rapidly after stimulation of M1 myeloleukaemic cells by IL-6 [77]. Later, MyD88 was found to be related structurally to the IL-1R family and, in particular, to TLRs [78]. Knock-out studies defined MyD88-dependent and MyD88-independent pathways of TLR responses [79] (Fig. 2).

Binding of PAMP to a particular TLR leads to the activation of TIR, and triggers a series of events leading to increased expression of pro-inflammatory genes. TLRs and the related interleukin 1 receptors (IL-1Rs) share considerable homology in their cytoplasmic regions, including a conserved homophilic domain of *c.* 200 amino-acids, known as the Toll-IL-1R domain. TLRs and IL-1Rs also share a common adaptor molecule, MyD88, which contains a Toll-IL-1R domain. After binding of a ligand, MyD88 is recruited to the TLR/TIR receptor complex, which is then joined by IL-1R-associated protein kinase-1 (IRAK-1), IRAK-4 and tumour necrosis factor receptor-associated factor 6 (TRAF6). IRAK-1 and TRAF-6 then dissociate from this complex and associate with another complex of transforming growth factor β-activated kinase (TAK-1) and TAK-1 binding proteins 1 and 2. TAK-1 is activated, which in turn activates the IκB kinase (IKK) complex. IKK-mediated phosphorylation of IκB leads to its ubiquitination and degradation, thereby unmasking the nuclear localisation domain of NF-κB [80]. After NF-κB translocation into the nucleus, NF-κB can activate multiple pro-inflammatory genes, including TNF-α, IL-1 and IL-6 [80].

Evidence is accumulating to indicate that different TLRs activate different signalling pathways and exert distinct biological effects. MyD88 is a shared adaptor molecule in IL-1, IL-18 and TLR signalling [81]. The importance of MyD88 as a component of TLR signalling is highlighted by the observations that MyD88-deficient mice have increased susceptibility to infection with pathogens including *Staphylococcus aureus* [82], *L. monocytogenes* [83], *Toxoplasma gondii* [84] and *Mycobacterium avium* [85]. In addition, MyD88-dependent responses have been found to be essential in streptococcal cell-wall-induced joint inflammation [86,87]. The MyD88-dependent pathway is essential for activation of the NF-κB and MAP kinases in response to all TLR ligands other than TLR4. However, the fact that each TLR ligand induces different patterns of gene expression in the same cell indicates that each TLR signal harbours MyD88-independent pathways (which may not activate either NF-κB or MAP kinases), in addition to the MyD88-dependent pathway [88].

Liu *et al*. [89] have demonstrated that MyD88 deficiency impairs pathogen clearance, but does not alter inflammation in *Borrelia*-infected mice [89]. Bolz *et al*. [87] observed that MyD88-deficient mice had extremely high numbers of *B. burgdorferi* in tissues when compared with wild-type litter-mates, and larger numbers of spirochaetes in tissues than did TLR2-deficient mice. This suggests that, in addition to TLR2, other pathways involving MyD88 play a significant role in host defence to *B. burgdorferi* [87].

In addition, a MyD88-independent pathway exists for stimulation by TLR4, and this leads to two major biological effects provoked by LPS, namely cytokine production and co-stimulatory molecule up-regulation. This MyD88-independent pathway may be linked to virus infection as it activates interferon regulatory factor-3 (IRF-3), leading to induction of IFN-γ and IFN-inducible genes, as observed for a range of infections. It has been reported that MyD88 plays a unique role in host defence, but it does not seem to be relevant for the development of arthritis in Lyme disease [87].

Type-I interferons, acting through their own receptors, activate the Janus kinases (JAK), which

in turn phosphorylate signal transducer and activator of transcription (STAT) proteins, leading to the transcriptional activation of other genes, including many that are required for effective antiviral defence and genes encoding proteins involved in the inflammatory response [90]. In the case of TLR4, association with at least three proteins occurs, namely MyD88, MyD88-adaptor-like associates (MaL, also known as Toll/IL-1R domain-containing adaptor protein; TIRAP), and the TIR domain containing adaptor-inducing IFN (TRIF). This suggests that additional MyD88-like adaptor molecules may associate with some TLRs to mediate additional specific responses [91]. TIRAP/MaL forms homo- or hetero-dimers with MyD88, and associates with IRAK-2, thereby leading to NF-κB activation [92,93]. An additional molecule involved in TLR-mediated signalling has now been identified as RICK/Rip2/CARDIAK [94,95].

ROLE OF CD14 IN *BORRELIA*-INDUCED INFLAMMATION

Vertebrates and invertebrates initiate a series of defence mechanisms following infection with bacteria or other microorganisms. The best-characterised example for innate recognition is the innate inflammatory response to LPS. In the case of LPS, the pattern recognition receptors are lipid-binding protein (LBP) and CD14. It is known that LPS binds first to LBP, a plasma lipid transfer protein that moves LPS monomers from aggregates or bacterial membranes to a binding site of CD14. LBP binds to LPS via lipid A [96], and thereby lowers the threshold stimulatory concentrations of LPS. However, *B. burgdorferi* lacks lipid A [44]. CD14, a leucine-rich protein, is expressed both in plasma (soluble CD14, sCD14) and as a glycosylphosphatidylinositol (GPI)-anchored protein on leukocytes (membrane CD14, mCD14) [97]. mCD14 lacks both trans-membrane and cytoplasmic domains [98]. Therefore, mCD14 is unable to transduce signals into the cytoplasm. It has been demonstrated that TLR4 acts as an additional signalling molecule in mammalian cells for the CD14-mediated transcriptional response to LPS. Acyl-modified lipoprotein of *B. burgdoferi* is a potent activator of monocytes/macrophages, B-cells and endothelial cells. The inflammatory signalling of *B. burgdorferi* lipoproteins is mediated by CD14 [6] and TLR2 [99]. According to Sellati *et al*. [100], the CD14-dependent signalling pathways used by LPS and spirochaetal lipoproteins/lipopeptides differ in at least two fundamental respects. In contrast to LPS, activation by spirochaetal lipoproteins is not facilitated by LBP or other serum components [100]. The absence of involvement of LBP in cell activation by lipoproteins and lipopeptides suggests that these amphiphilic compounds either interact with CD14 as aggregates, or that monomers bind to CD14 unassisted by a serum intermediary such as LBP [100]. This type of LPS activation has also been reported in *S. aureus*. Cell-wall extracts of *S. aureus* stimulate human PBMCs in a CD14-dependent, but LBP-independent, pathway [101].

LBP has been shown to be expressed in hepatocytes, alveolar epithelial cells and intestinal cells. Thus, LBP is apparently present in compartments of the organism exposed to larger quantities of microorganisms, and may play a key role in early recognition of pathogens. LBP is reported to enhance LPS responsiveness by transferring LPS monomers out of LPS aggregates to a binding site of CD14 [102]. It has been demonstrated that activation of monocytes and human umbilical vein endothelial cells by spirochaetal lipoproteins and lipopeptides is enhanced by CD14, particularly at low doses [6,100]. The dependence on CD14 was lost at higher concentrations of lipoproteins [6]. It seems apparent that spirochaetal lipoproteins follow a CD14-independent stimulatory pathway at higher concentrations. Elevated levels of soluble CD14 (sCD14) exist in the synovial fluid of patients with *B. burgdorferi* infection in their affected joints, compared with normal levels in serum [103]. CD14 is also involved in cellular responses to triacylated and diacylated lipopeptides. Lipopeptides display a pattern of recognition via TLR2, in combination with TLR1 and/or TLR6, related to their different degree of acylation. There are similarities in the signalling pathways of LPS and lipoproteins, which indicate that they converge by stimulation of the nuclear translocation factor NF-κB [104].

CONCLUSIONS

Lyme arthritis, the most common manifestation of late Lyme disease, has been associated with the presence of *B. burgdorferi* in the joint [105,106]. The

abundantly expressed borrelial lipoproteins are intricately involved in the pathology of Lyme disease. Targeting TLRs, the lipoproteins possess potent stimulatory properties for inflammatory host cells, e.g., neutrophils, mononuclear cells, endothelial cells, etc., that are present in affected joints [99]. Thus, an understanding of the molecular basis of the signalling events elicited by lipoproteins should lead to a greater understanding of inflammation in Lyme arthritis.

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